THE REGENERATION OF THE PHARYNX IN PLANARIA MACULATA.

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In a paper on *Planaria maculata*, published in 1898, Morgan called attention to an important feature of the regeneration of the pharynx, namely, that in regenerating pieces which were cut anterior to the old pharynx, the new pharynx forms "at the edge of the old tissue, but lying for the most part within the *new* material," while if the cut were made posterior to the pharynx the new organ forms in the anterior end of the *old* tissue. During the next two years there appeared a number of papers describing experiments on the power of regeneration in planarians, but giving no further knowledge of the development of the pharynx except that to be derived from general statements of the various positions it may assume in the regenerating pieces. More recently a paper by Bardeen on the physiology of *Planaria maculata* has taken up the formation of the pharynx in this species and given a somewhat detailed account of it.

In studying the origin and growth of the new pharynx, I wished to find out if there were any marked difference between its formation in the *new* tissue of the anterior piece and in the old tissue of the posterior piece, since the conditions for regeneration seemed so unlike in the two cases. At the same time I was interested in observing in the posterior pieces what might be the influence of the reproductive organs when present in the region where we should expect the pharynx to appear, and if, under these conditions, the already existing cavities and external opening can be made use of. In this connection I examined series of regenerating posterior pieces in which the cut had been made (1) just posterior to the pharynx, but at a time of year when the reproductive organs have disappeared; (2) anterior to these organs when they are fully developed; and (3) posterior to them.

The greater part of the material for my experiments was collected from ponds near Woods Hole during the months of June, July, and August, and was preserved for study in the following winter. Later in the year more planarians were found near Bryn Mawr, and were kept in aquaria until needed. Each worm was cut into three pieces, of which the middle, containing the pharynx, was rejected, and the anterior and posterior pieces were allowed to regenerate. The latter were killed at intervals of twelve hours for about eight days, at the end of which time those remaining had begun to feed. Where it seemed advisable, series were killed at closer intervals, but as a rule this was not necessary. The pieces remained in corrosive acetic for about ten minutes; after cutting they were stained on the slide in Delafield's hæmatoxylin, and then dipped for a few seconds into a strong solution of aniline orange. The latter differentiates the endoderm, muscle bands, yolk cells, and the lining of the reproductive system.

Soon after the worms are cut, the edges of the cut-end draw together and matter from the digestive tract and loose parenchyma tissue collect about the injured region. Within a few hours the surface opens out again, until only a slight depression marked by a dark line of pigment is noticeable. In the mean time parenchyma cells collect at the cut surface and form a narrow sheet across it, while cells from the margin of the uninjured epithelium push out and cover the new growth. This overgrowth of epithelium is comparatively rapid, as the cells in this region flatten out and cover individually a much larger area. By the end of the first day the tip has become rounded and the anterior end is covered with an epithelial layer; later, with the increase in their number, the flattened cells resume their columnar form.

The parenchyma of the normal planarian appears on examination as an ill-defined mass filling in the spaces between the organs. Its protoplasm stains very slightly, but the tissue is conspicuous owing to the many deeply colored nuclei that are present in it. These nuclei are occasionally seen in small groups, but they are usually scattered and separated from each other by the loose spongy tissue. Among the parenchyma

cells are found other cells which are specialized in function,—large rhabdite cells, lying just below the ectoderm and showing the rhabdites buried in the protoplasm, and the mucous cells, staining an intense blue; also, along the digestive tract, a few large granular cells for which Bardeen has suggested a digestive function. In addition, I have noticed some large cells staining with aniline orange, which seem to contain some such material as yolk. Whether this is a fourth kind or merely the digestive cells I have not been able to decide. There are also present elongated connective-tissue cells.

Although the majority of the parenchyma cells show little protoplasm, scattered through the tissue of the normal uninjured animal there are a good many cells whose nuclei resemble those of the ordinary parenchyma, but which have gathered about them an irregular mass of granular protoplasm. As many of these are in various stages of division, while I have found no division among the cells with less protoplasm, it is probable that the accretion of protoplasm is the usual forerunner of division in the parenchyma cells, and that the number of the latter is constantly increasing even in normal planarians. While the number of these dividing cells differs individually, they are often very numerous and are confined to no one region of the body, except that they are more rare in the extreme tips. The dividing cells stand out very distinctly as they are larger and seldom have other cells near them.

After the animals are cut, the amount of division increases somewhat throughout the whole body, but very markedly near the injured surface. Cells also migrate down into this region and form there a thickening which rapidly enlarges. That migration takes place is indicated by the long trails of protoplasm that give to the cells a bipolar appearance with the axes directed towards the growing mass (Fig. 1), while in the normal planarian there is no such effect and the cells are more irregular and rounded in outline. After the thickening has reached a certain degree of concentration there is rarely any cell division in the proliferated mass, but mitotic figures can frequently be found in the region near by, where the cell mass is less dense. Cells also continue to migrate into the new part and

add to the new growth. The number of cells in the parenchymatous tissue of the body itself is also usually much increased as the nuclei throughout continue dividing, and by the time the pharynx thickening appears, which is in the course of the third day, the tissue is filled with cells conspicuous on account of their greater amount of protoplasm.

I shall consider first the growth of the pharynx in the anterior piece and then that in the posterior piece. By the time the proliferation of cells in the regenerating posterior tip is quite marked, a slight shift in the direction taken by some of the cells denotes the beginning of the new pharynx. The cells now collect just on the line between the old and new tissue at the posterior end of the digestive tract, which has rounded off, and usually has begun to bud out side branches. As is true of the growing end, so here, when the thickening becomes marked, no division of cells can be found in it, but in the less dense tissue around it there are many dividing cells and apparently considerable cell migration towards the new growth. The collection of cells stains very deeply in comparison with the old tissue near by, due probably to the greater amount of protoplasm in this region.

The thickening for the pharynx grows until it is quite large, and then the split for the pharynx chamber begins. This opens up very rapidly as a narrow cavity running across the base of the thickening and up on the sides, being usually more advanced on the ventral than on the dorsal side. It is at first irregular in outline, but the cells along the edge soon flatten out into a thin lining epithelium. As development proceeds, cells are added at the anterior end of the new pharynx, while there is no division in the compact mass of cells already collected (Fig. 2). About the fourth day the lumen of the pharynx itself appears in the center of the proliferation, due to an elongation and pulling apart of cells which become larger and thin walled and form the lining of the pharynx. This cavity gradually enlarges posteriorly to open into the pharynx chamber, and anteriorly to unite with the wall of the digestive tract, which then breaks through to join with it. The new region has in the mean time steadily increased in

length, and the branches of the intestine have pushed back into it, while the central digestive cavity has also somewhat enlarged. Shortly after the lining of the lumen has formed, the muscle bands of the pharynx differentiate. They arise from cells just between the two epithelial layers; they elongate and undergo some change in their constitution, since they stain with aniline orange very shortly after the lengthening process has begun. The change is first visible in the region where the pharynx is continuous with the body of the animal

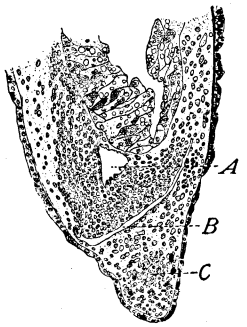

FIG. 2.

FIG. 1.

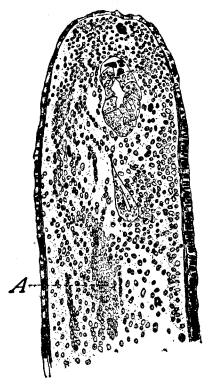

FIG. 3.

FIG. 1. — Large parenchyma cells migrating towards regenerating region.

FIG. 2. — Pharynx in anterior piece at close of fourth day. The lumen has just appeared. *A*, lumen; *B*, lateral digestive branch; *C*, pharynx chamber.

FIG. 3. — Pharynx in posterior piece at close of third day. The lateral digestive branches have met, and the pharynx thickening (*A*) is conspicuous. There are many dividing cells present.

and extends gradually posteriorly. So far as I could determine, the circular muscles develop before the longitudinal, but if so, the difference in time is very slight. In the course of the fifth or sixth day the pharyngeal chamber opens to the exterior by an ectodermal invagination from the ventral surface, and shortly afterwards the animal begins to feed.

The shape and size of the pharynx vary comparatively little after the inner lumen appears. At first, before the formation of this cavity, the shape is slightly pointed, but it soon becomes more regular and assumes the form of the normal pharynx, except for its length, which is only about once and a half or twice the breadth. The addition of cells at the anterior end

does not continue after the structure is well organized, and the latter part of the time needed for the regeneration of this organ is occupied in changing the parenchyma cells into the different tissues. Up to the time when the muscles appear, the cells still remain crowded together and show by their deep color the presence of unusually dense protoplasm. After the muscles are well formed the tissue assumes a less dense appearance, and this change arises apparently at the same time in the cells throughout the new tissue. Deeply staining masses, as of mucous, can also be seen, so that the tissue soon resembles that of the normal worm.

The process of formation of a new pharynx in the old tissue of the posterior piece seems to be very similar, and equally simple during the nine months of the year when the reproductive organs are absent. The overgrowth of the cut surface and the increase in the number of dividing cells is just as was found in the anterior piece. The pharynx thickening, however, appears about twelve hours later than in the former case, *i.e.*, about the end of the third day, and is usually noticeable shortly after the two branches of the digestive tract have joined. But that the pharynx is formed with relation to the "axial gut," as Bardeen states, I am not prepared to say, as occasionally a case is found in which the branches have not met, although a thickening is present, and often in a slide showing a conspicuous gathering of cells the union between the intestines has barely taken place. The pharynx forms a short distance posterior to the cut, in the old tissue. It is first made visible by the shifting of the axes of some of the cells, which now direct themselves toward the new point of activity, and the thickening increases rapidly (Fig. 3). The cavities open up just as in the anterior piece, but the chamber is apt to be much longer and more irregular in outline than in the other case. Later the enlarged cavity rounds off so that the chamber resumes its normal size. The central cavity of the digestive tract enlarges in a posterior direction, and at the same time the pharynx thickening is added to by cells at the anterior end; thus, by the time the pharynx is ready to open up, the original space between it and the

digestive tract is bridged over (Figs. 4, 5). The appearance and development of the lumen, muscle bands, and normal spongy tissue is identical with that in the anterior pieces, and the posterior piece is also ready to feed in seven days.

Summing up these results, it appears that the regeneration of the pharynx in the new and in the old tissue is much the same, (1) as to the origin and migration of the cells, (2) as to the formation of the two cavities (the chamber and the lumen of the pharynx), and (3) as to the differentiation of the cells into the normal tissues. They differ (1) in the length of time that elapses before the pharynx thickening shows itself (about twelve hours more being required for its appearance in the old tissue than in the new), (2) in the position of the developing

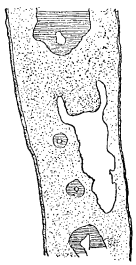

FIG. 4.

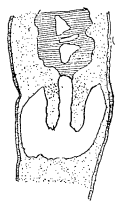

FIG. 5.

FIGS. 4, 5. — Stages in development of pharynx in posterior piece.

pharynx with regard to the central digestive cavity, and (3) in the size of the pharyngeal chamber as shown by its early irregularities in the posterior piece. The last two differences are probably due to the disposition of the digestive branches in the two cases.

In the early part of the summer the reproductive organs of this species are fully developed, and regeneration under these conditions is especially interesting. The genital apparatus lies for the most part in the region just between the pharyngeal and genital pores, so that when an animal is cut just posterior to the pharynx these organs occupy the region where the new pharynx should develop. Under these conditions there is no sign of the growth for the new pharynx until towards the end of the fourth day, when a small collection of cells can

be found in a ventral but not necessarily median position. Also this mass is usually at a normal distance from the fused intestine. Morgan (1900) has noted this delay in the formation of the pharynx when the worm is cut anterior to the genital pore at this time of year (July 2) and finds about twelve days instead of seven required for complete regeneration. His later experiments, begun July 31, gave different results, but this was probably due to the degeneration of the reproductive organs between these two dates, as he found that the time required for this set of pieces to become complete animals was only seven days.

This delay may be due to the scarcity of parenchyma cells in this region, which is almost completely filled by the tissue of the genital organs. When these organs are fully present the parenchyma cells collect as shown in Fig. 6, migrating principally from the anterior end into the region of parenchymatous tissue that lies just ventral to the penis. In this drawing are shown in the tissue along the edge of the genital organs certain irregular breaks that seem to appear at the beginning of the degeneration of these structures. The pharynx forms ventral to such cavities, and the split for the pharynx chamber is at first entirely independent of them (Figs. 6, 7). Later the wall between may break down, and thus greatly enlarge the chamber, but if so it is a secondary process; there is also a tendency towards degeneration in the thin line of tissue between the pharynx and reproductive chambers, as shown by the dotted line in Fig. 7. In no case, however, does the old genital pore become the new pharyngeal pore.

As the genital organs degenerate, which I have found takes place during the last two weeks in July and the beginning of August, the region in which the pharynx appears becomes quite irregular. The only feature that seems at all constant is the relative distance between the cross-branch of the intestine and the pharynx thickening. That this is true may be seen by a study of its position with relation to that of the genital pore. The main genital duct opening to the exterior persists longer than any other part of these structures, but its presence

seems to have little or no influence on the position of the new pharynx. The latter may appear far anterior to it, posterior, or, as in Fig. 8, it may assume an eccentric position and lie on the same level; in this case the duct and pore are shown by dotted lines, as the pharynx lies about eight sections to the side of the median line, *i.e.*, of the genital pore. Thus the new pharynx can be found in all possible positions, and the varia-

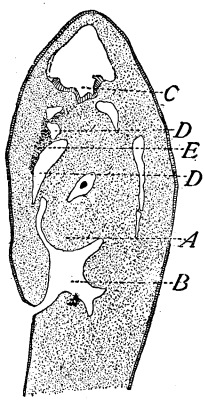

FIG. 6.

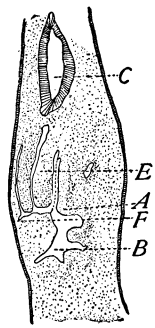

FIG. 7.

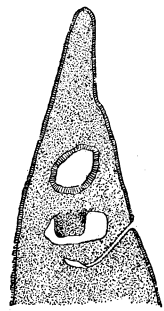

FIG. 8.

FIGS. 6, 7. — Development of pharynx during presence of reproductive organs. *A*, penis; *B*, genital chamber; *C*, digestive tract (in 6 the wall has broken down between it and the degenerating cavity); *D*, cavities due to degeneration of reproductive organs; *E*, pharynx; *F*, position of pharyngeal pore.

FIG. 8. — Position of regenerating pharynx with respect to genital duct and pore. The new organ appears to one side of the pore but on the same level. The dotted lines indicate direction of duct in following sections.

tions, such as lateral, ventral, and oblique, should be noted in connection with any hypothesis of the relation of the pharynx to the axial gut.

As regards the process of regeneration in the posterior pieces of worms cut behind the genital pore when the reproductive organs were believed to be fully developed, the process of formation seems identical with that which takes place during the absence of these organs. Consequently there is no need to describe what takes place.

I wish to express my indebtedness to Prof. T. H. Morgan, under whose direction this work has been done.

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